

between HBV replication and the expressions of transforming growth factor (TGF)- β 1 and insulin-like growth factor-II (IGF-II) in tissues of hepatocellular carcinoma (HCC).

Methods: Liver HBV-DNA was detected by *in situ* molecular hybridization technique, and the expressions of TGF- β 1 and IGF-II were detected by the immunohistochemistry, and TGF- β 1 mRNA and IGF-II mRNA were amplified by nested-PCR assay in HCCs and their self-control non-cancerous tissues. The relationship was investigated between TGF- β 1 or IGF-II expression and HBV replication or their clinical pathological characteristics.

Results: The stronger expressions (83.3%) of TGF- β 1 and IGF-II were found, and the incidences of TGF- β 1 mRNA and IGF-II mRNA were 100% in HCC tissues. A significant difference was presented between in HCC tissue and in non-cancerous liver tissues ($P < 0.01$). The positive rate of TGF- β 1 in HCC was correlated to tumor differentiation, but neither to tumor size nor numbers ($P > 0.05$). The levels of TGF- β 1 and IGF-II expression were significantly associated with HBV replication with higher HBV-DNA-positive HCC (94.7%) than that in HBV-DNA-negative group.

Conclusion: TGF- β 1 and IGF-II in HCC are overexpressed and associated with hepatic HBV replication and differentiation degree of HCC.

PP-104 Chronic hepatitis B: molecular, epidemiological and clinical features in northwest and central Russia

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Objectives: To reveal the HBV genotype distribution and clinical differences between different genotypes in patients with chronic hepatitis in 2 regions of Russia, to determine the structure of HBV DNA fragments responsible for resistance to treatment.

Methods: HBV genotyping was carried out by original PCR method. Sequencing with original primers was performed. Real time PCR was used to determine viral load in patients' blood and liver biopsies. Disease severity was evaluated by clinical-laboratory markers and morphology study.

Results: 325 patients with chronic hepatitis B (Mean age - 34 years. Female to male ratio - 2:1) were observed. HBV genotype was determined in 294 patients from Saint Petersburg region and 31 patients from Central part of Russia (Perm city). HBV D genotype was revealed in 96% of cases in North West and in 67% of cases in Central Russia. In the rest of cases A genotype was determined. More severe disease with necro-inflammatory infiltration and fibrosis progression was diagnosed in patients with HBV A genotype. Some patients from this group had hepatocirrhosis. 95% of patients with HBV D genotype were HBeAg-negative. HBV polymerase gene fragment sequencing from 28 patients revealed high variability of viral genome and YMDD-motif characteristic for wild type (lamivudin susceptible).

Conclusion: HBV genotypes A and D are circulating in North West and Central Russia. The percentage of prevalent D genotype is much higher in the North West region. Prevalence of mutant D genotype HBV enforces to include nucleoside analogues in therapeutic course of HBV chronic patients.

PP-105 Development of an ELISA kit for detection of HBsAg in human serum

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Background: Hepatitis B virus, the cause of serum hepatitis, is classified as a hepadnavirus. HBV is responsible for acute and chronic infections and may ultimately lead to cirrhosis or primary hepatocellular carcinoma. HBV have various antigens, the important of which is surface antigen or HBsAg.

The particles containing HBsAg are antigenically complex. In contrast to the other HBV antigens, HBsAg is an important diagnostic marker of an active hepatitis B infection. Emphasis of this study is to develop a direct sandwich ELISA method for detection of HBsAg in the human serum.

Methods: In summary, the surface of well of microplate was coated by mouse monoclonal anti-HBs with the concentration of 1 J.1g1mTl.he serum or plasma sample added to the wells of microplate after they had been saturated by BSA. Then, diluted anti-HBs-HRP (1/8000) was added to each well which was able to connect to the trapped HBsAg. The final colorimetric detection of HBsAg is performed by adding a solution of substrate of peroxidase enzyme to each well. The color intensity is directly proportional to the concentration of HBsAg in serum.

Results: The sensitivity and specificity of the developed method was studied on 1350 serum samples. The results indicated a 96% sensitivity and 98.8% specificity. The precision of this method was determined by the %CV for inter-assay and intra-assay.

Conclusions: We can develop a direct sandwich ELISA method for detection of HBsAg in the human serum with 96% sensitivity and 98.8% specificity.

PP-106 Analysis of lymphocyte subsets of peripheral blood in patients with acute self-limited hepatitis B

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Objectives: To investigate changes and significance of lymphocyte subsets (T lymphocytes, B lymphocytes, NK cells and T cell subsets) of peripheral blood in patients with acute self-limited hepatitis B (AHB) with changes.

Methods: By flow cytometry (FCM), immune cells of peripheral blood were compared among 23 cases of self-limited acute hepatitis B patients, 36 patients with chronic hepatitis B (CHB) and 32 healthy controls; CD4⁺/CD8⁺ and ALT were monitored dynamically, meanwhile the relation between T lymphocyte subsets and ALT were explored.

Results: The level of CD3⁺ T cells (75.02 \pm 8.71%), CD3⁺CD4⁺T cells (43.32 \pm 6.73%) and the ratio of CD4⁺/CD8⁺ (2.35 \pm 0.51%) of AHB have significantly increased compared to CHB group (62.48 \pm 11.33%, 33.07 \pm 9.67%, 1.14 \pm 0.31% respectively) and healthy group (64.00 \pm 11.54%, 34.41 \pm 7.53%, 1.41 \pm 0.61% respectively); Dynamic monitoring of CD4⁺/CD8⁺, ALT, CD4⁺/CD8⁺ had an increased trend, accompanied by lower ALT. And CD4⁺/CD8⁺ had no significant relation to HBV-DNA for HBV-DNA positive AHB.

Conclusion: Immune status of AHB, compared to CHB and healthy controls, were significantly different and changes of T lymphocyte subsets were related to progress of disease.

PP-107 Up-regulation of expression of B7-H1 and its receptor PD-1 on PBMC by HBeAg

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Objectives: To study HBeAg does impact on molecule expression of B7-H1 and PD-1 as well as TLR2 on PBMC.

Methods: PBMC from different infectious status of CHB patients were stimulated by recombinant HBeAg, and expression of B7-H1, PD-1 and TLR2 on PBMC before and after stimulation as well as changes in lymphocyte subsets were quantitatively analyzed on FACS, changes in expression of PBMC surface receptor above were further observed by using blocked method of HBeAb, in order to confirm HBeAg-specific function, while analyzing growth and decline of cytokines in culture supernatant before and after stimulation.

Results: When PBMC from HBeAg-negative CHB patients and